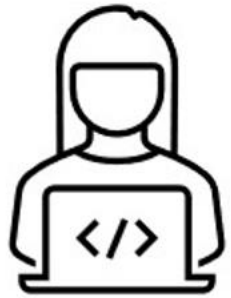
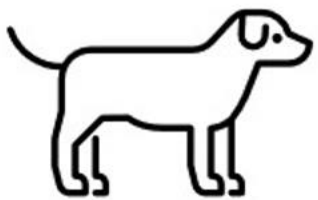




OIE Standards on Rabies Diagnostics

Changchun Tu

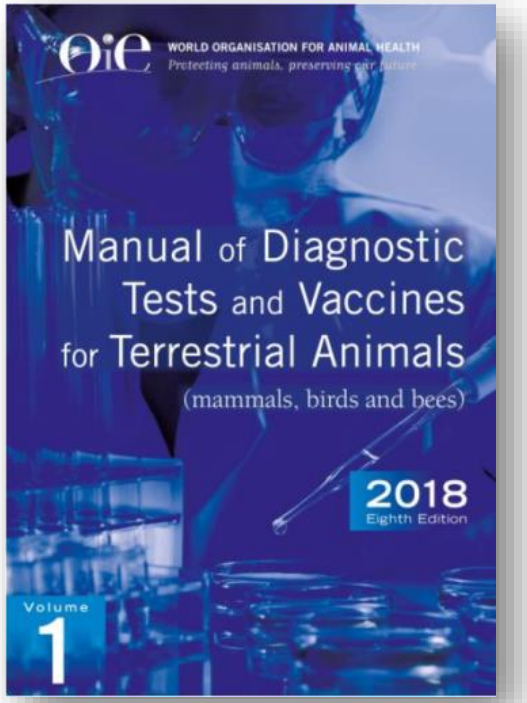
OIE Reference Laboratory for Rabies
Changchun Veterinary Research Institute, China

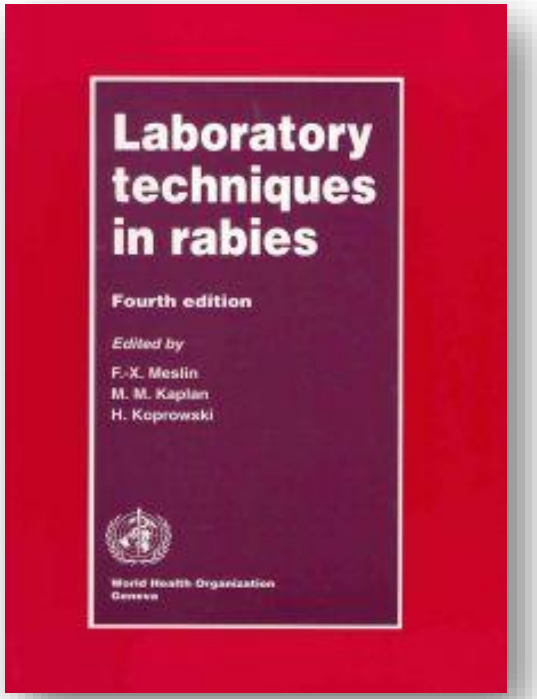
OIE SEA Sub-Regional Virtual Workshop on OIE international standards supporting self-declaration of freedom from rabies, and endorsement of official control programme

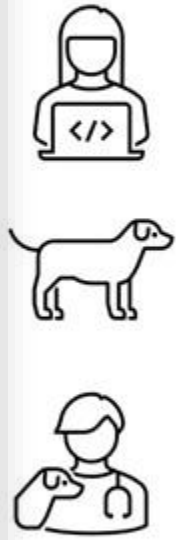
6-8 July 2021

Rabies Diagnosis

1. Sample collection
2. Shipment
3. Laboratory tests
   (1) Detection of viral antigen
      a. **DFA**
      b. dRIT
   (2) Detection of live virus
      a. Cell culture test (RTCIT)
      b. Mouse inoculation test (MIT)
   (3) Molecular techniques
      a. **Conventional RT-PCR**
      b. **real-time PCR**
4. Serology tests
   (1) virus neutralisation: FAVN and RFFIT
   (2) **antibody ELISA**

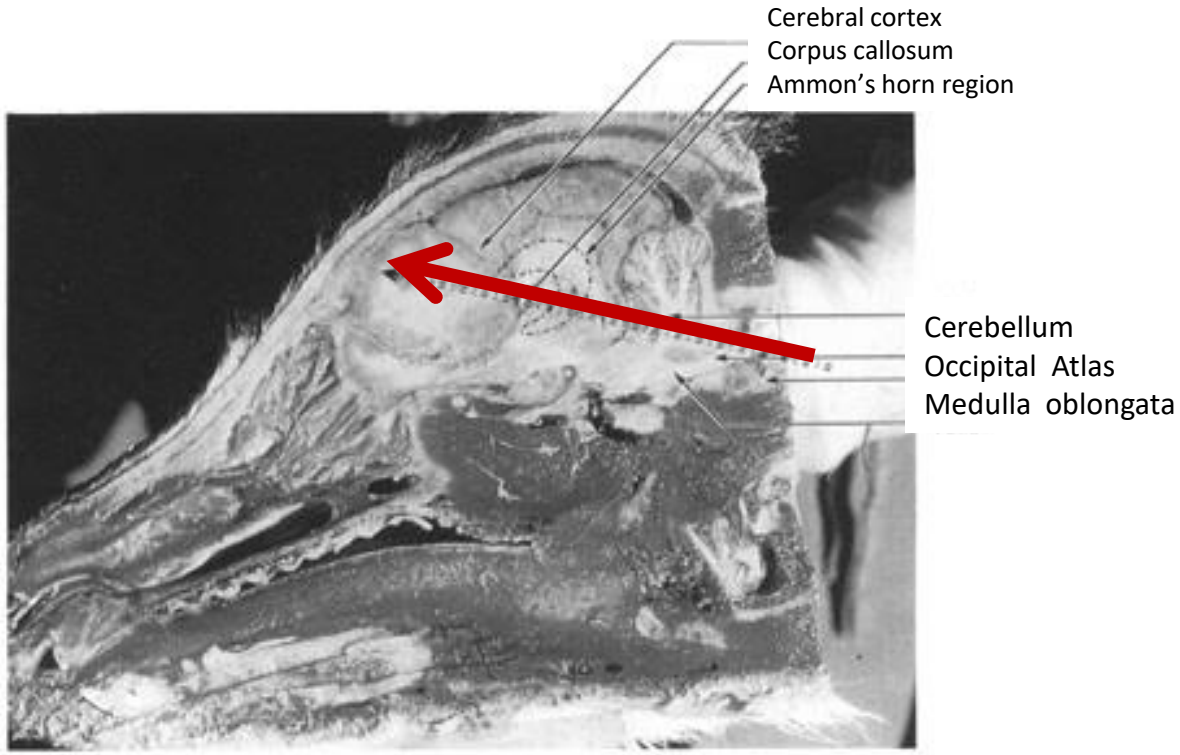


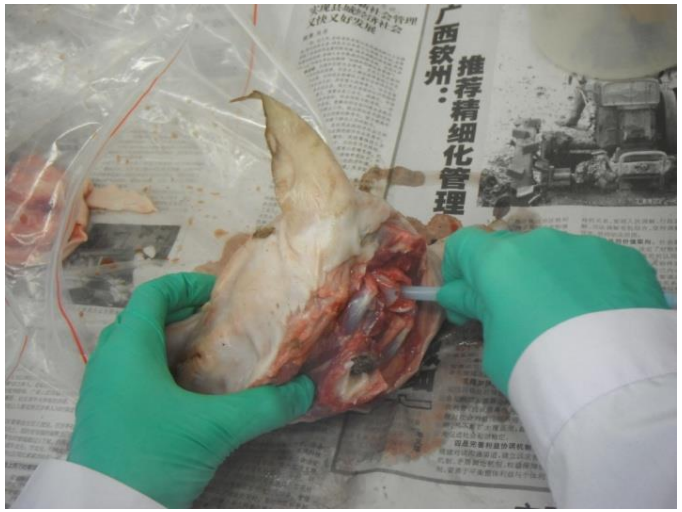




1.1 Collection of Brain Samples

Straw method: occipital foramen route for brain sampling

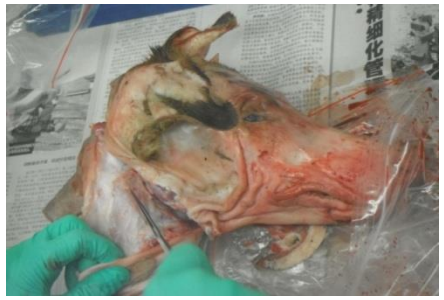


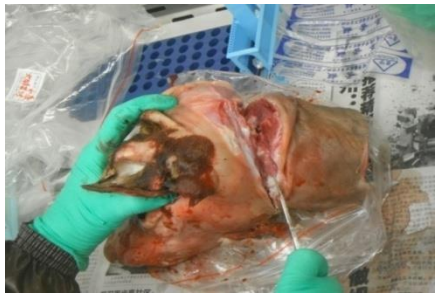


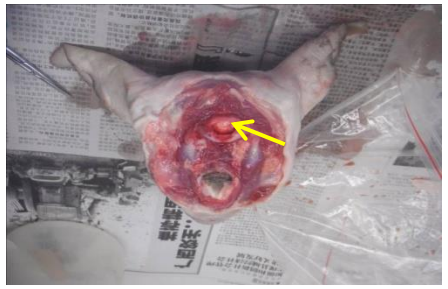
Insert the straw into the occipital foramen towards one eye. Samples are taken from the medulla oblongata, the base of the cerebellum, the hippocampus and the cerebral cortex.

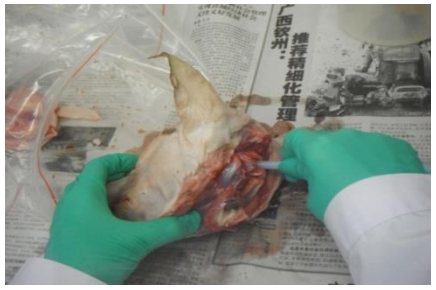
Collection of brain samples

Occipital foramen route for brain sampling



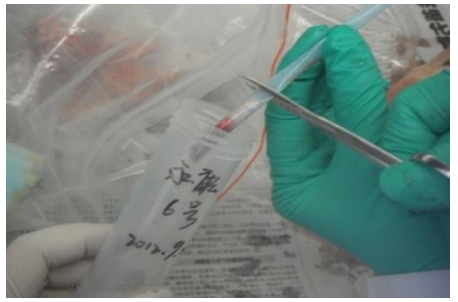


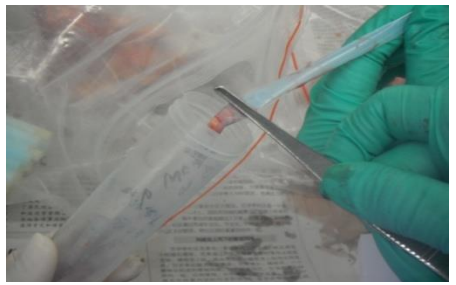




Expose the occipital foramen

Insert plastic straw





1. Transfer the tissues into tubes, and transit to the laboratory in ice container
2. Refrigeration during transit is not needed If the sample is in glycerol

1.2 Shipment of Samples

Journal of Virological Methods
Volume 140, Issues 1–2, March 2007, Pages 174-182

Use of filter paper (FTA®) technology for sampling, recovery and molecular characterisation of rabies viruses

The stability of viral RNA and the inactivation of infectivity make the FTA® cards useful for the storage, transport, collection and subsequent molecular analysis of viral rabies RNA.

- Impregnated with chemical forma that lyses cell and denature protein upon contact.
- The chemical forma can inactivate virus after a 2 h contact with FTA® cards.
- Widely used for bacteria, viruses, variety of tissues in any form
- Protecting NA and denaturing protein make FTA useful for PCR, **not for antigen detection (such as DFA, etc).**
- RNA could be stored 35-48 days on FTA® cards.
- International shipment is possible without import permit.

1.3 Laboratory tests

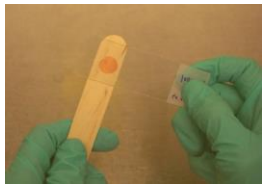
Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification						
DFA (antigen detection)	+++	n/a	+++	+++	+++	n/a
dRIT (antigen detection)	+++	n/a	+++	+++	+++	n/a
ELISA (antigen detection)	+	n/a	+	+	+	n/a
Cell culture (virus isolation)	+	n/a	+++	+++	+++	n/a
MIT (virus isolation)	n/a	n/a	+	+	+	n/a
Conventional RT-PCR (RNA detection)	+++	n/a	+++	+++	+++	n/a
Real-time RT-PCR (RNA detection)	+++	n/a	+++	+++	+++	n/a

1.3.1 Immunochemical identification of rabies virus antigen

i) Direct fluorescent antibody (**DFA**) Test

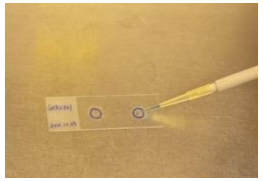
- Golden standard of OIE and WHO
- suitable for brain tissue; cell monolayers
- simple with high sensitivity and specificity
- 98-100% accuracy
- Requisite for rabies laboratory
- National Standard of China (GB/T18639-2002)
- Need fluorescent microscope, trained technicians

impression



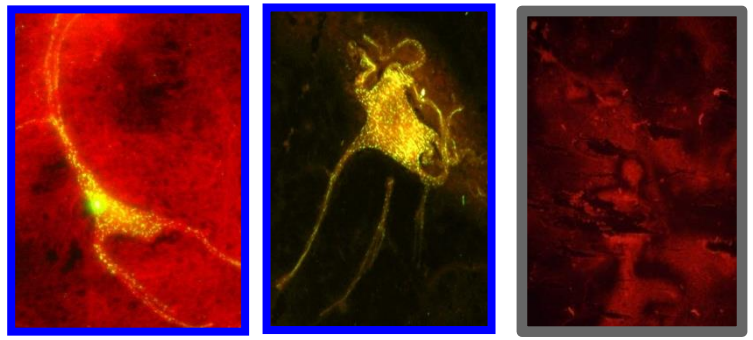
fixation

staining

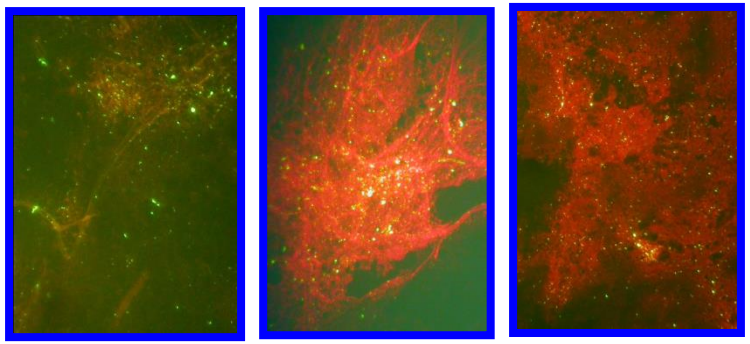


observation

Positive **Negative**



Bright apple green inclusions in neuron cells

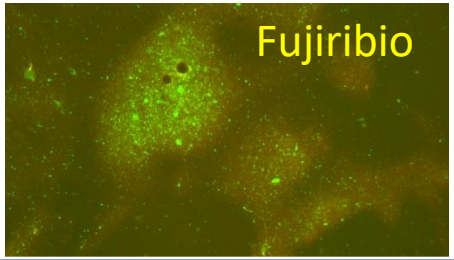


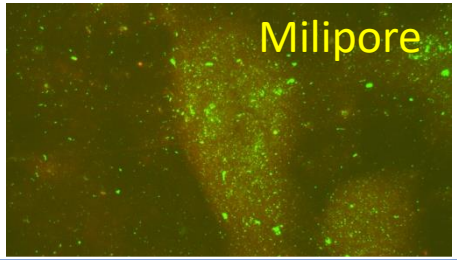
dog cow sheep

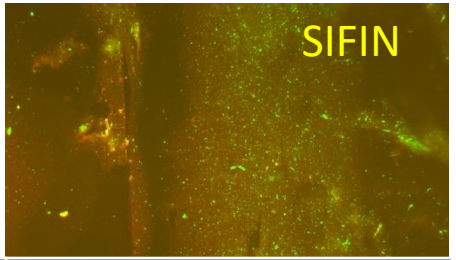
i) Direct fluorescent antibody (DFA) Test

Comparison of various anti-rabies conjugates

RABV







	Fujiribio (USA)	Milipore (UK)	SIFIN (Germany)
RABV	++++	++++	++++
LBV	+	++	++++
MOKV	+	++++	++++
DUVV	+	++	+
EBLV-1	+	+++	+++
EBLV-2	++	+++	+
ABLV	+++	++++	++

Scores:
- 4 ++++: very bright green fluorescence
- 3 +++ bright green fluorescence
- 2 ++ dull green fluorescence
- 1 + dim but detectable green fluorescence

ii) Direct Rapid Immunohistochemistry Test (**dRIT**)

- Has the same specificity and sensitivity as FAT
- Used as an alternative to FAT
- Use only light, not fluorescent, microscope
- Suitable for field work

Positive results based on the presence of magenta inclusions on a blue neuronal background.

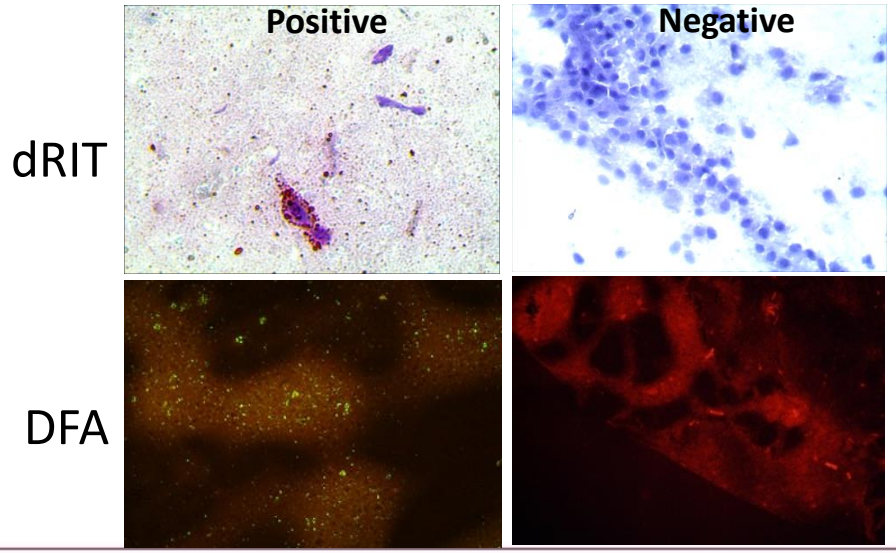


Table 2. Number of Tanzanian brain samples processed by dRIT and DFA for different animal species*

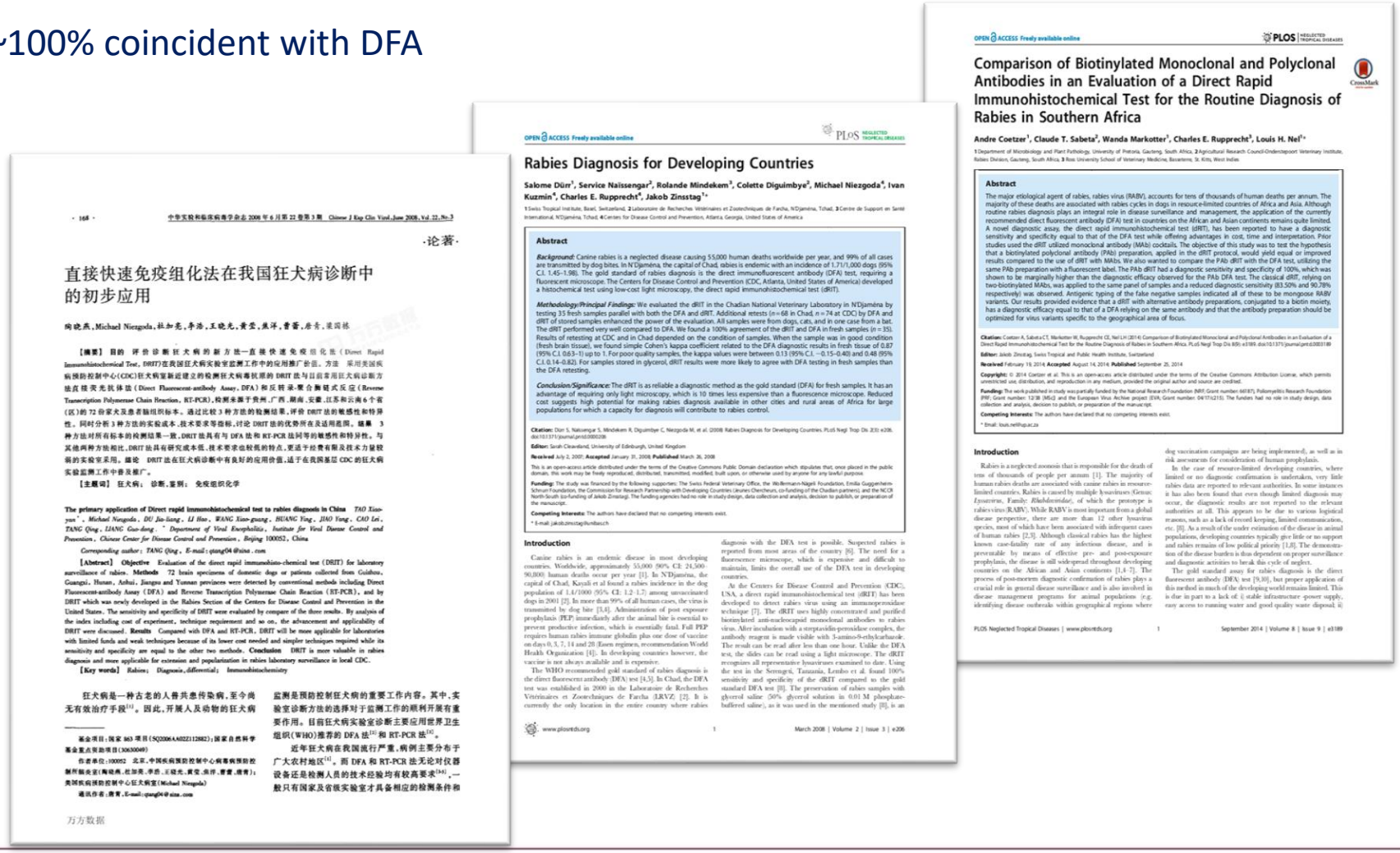
Species	No. brains examined†
Domestic dog	73 (39)
Domestic cat	7 (3)
Cow	8 (7)
Goat	6 (5)
Livestock‡	1 (1)
Aardwolf (Proteles cristatus)	1
African civet (Civettictis civetta)	2
Banded mongoose (*Mungos mungo*)	2
Slender mongoose (Herpestes sanguineus)	3
Dwarf mongoose (*Helogale parvula*)	2
White-tailed mongoose (*Ichneumia albicauda*)	8 (1)
Mongoose‡	2
Black-backed jackal (*Canis mesomelas*)	3
Bat-eared fox (Otocyon megalotis)	8
Black-backed jackal/bat-eared fox‡	2 (1)
Cheetah (Acinonyx jubatus)	3
Small-spotted genet (*Genetta genetta*)	7 (1)
Lion (Panthera leo)	6
Serval (Felis serval)	1
Spotted hyena (*Crocuta crocuta*)	12 (1)
Striped hyena (*Hyaena hyaena*)	1
Zorilla (Ictonyx striatus)	1
Total domestic	95 (55)
Total wildlife	64 (4)
Total	159 (59)

*dRIT, direct immunohistochemical test; DFA, direct fluorescent-antibody assay.
†The number of rabies-positive samples is shown in brackets.
‡Species not definitively identified.

(Lembo, et al. Evaluation of a Direct, Rapid Immunohistochemical Test for Rabies Diagnosis. ***Emerg Infect Dis***, 2006)

ii) Direct Rapid Immunohistochemistry Test (**dRIT**)

~100% coincident with DFA



直接快速免疫组化法在我国狂犬病诊断中的初步应用

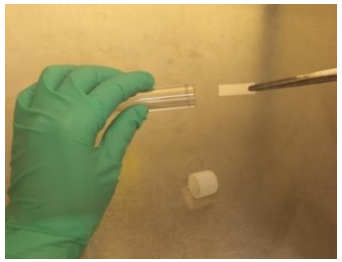
Rabies Diagnosis for Developing Countries

Comparison of Biotinylated Monoclonal and Polyclonal Antibodies in an Evaluation of a Direct Rapid Immunohistochemical Test for the Routine Diagnosis of Rabies in Southern Africa

WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future

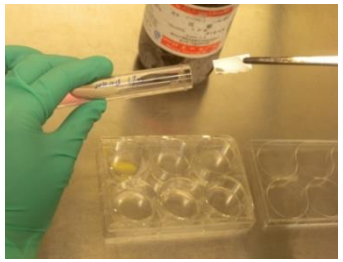
1.3.2 Virus isolation

i) Rabies Tissue Culture Isolation Test (RTCIT)

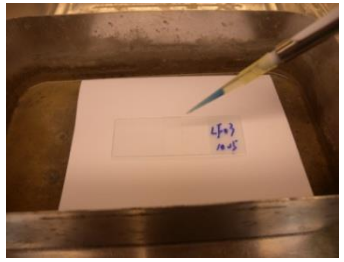


Place a coverslip into the inclined plane

Add brain suspension sample to the cell



Fix the plane

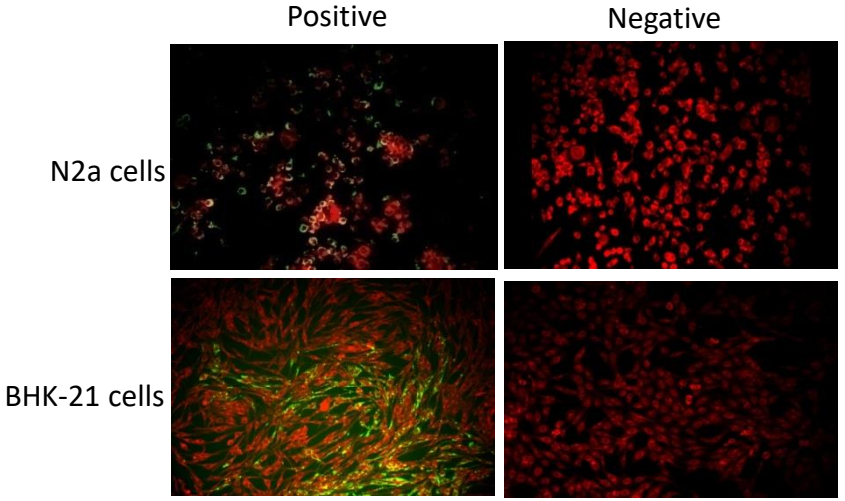


Add antibody conjugate

Incubation

Observation

- OIE and WHO standard
- high sensitivity and specificity
- used for virus detection and isolation
- National standard of China: GB/T18639-2002
- disadvantage: time consuming, BSL3 facility
- N2A and BHK-21 cell lines

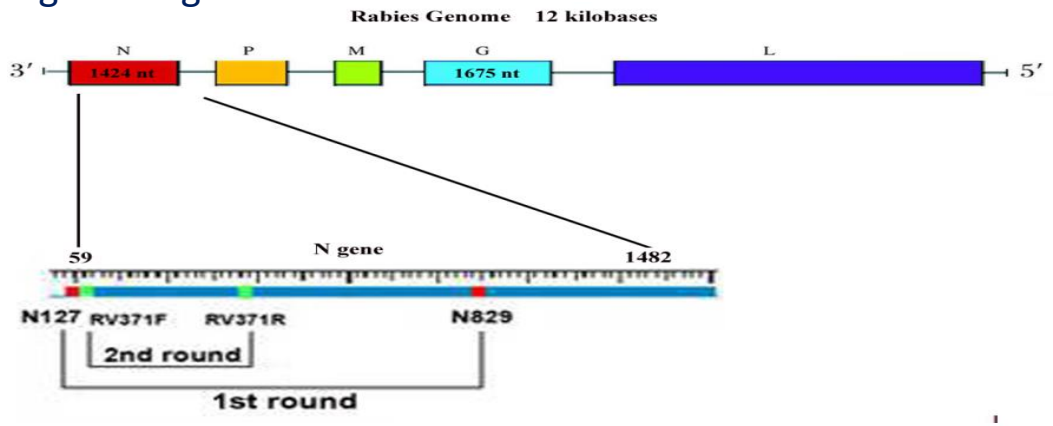


ii) Mouse inoculation test (MIT) is less used due to animal welfare.

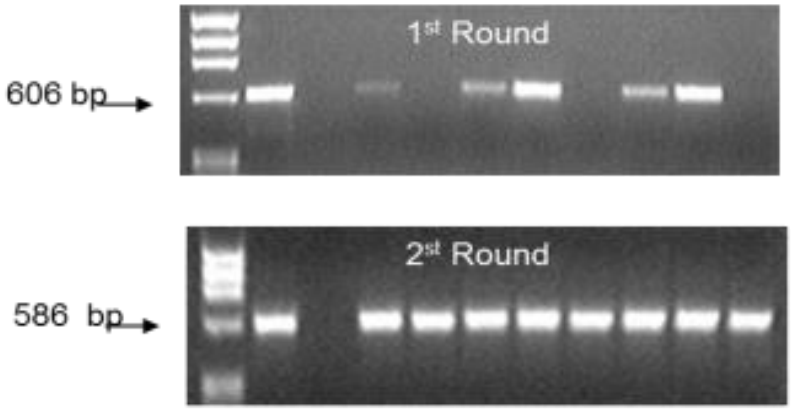
1.3.3 Conventional RT-PCR

- OIE and WHO reccommended
- high sensitivity and specificity
- Genotyping: phylogenetic analysis
- Pan-lyssavirus
- Disadvantage: unable to differentiate; false positive or false negative; very stringent quality control (partition of room).

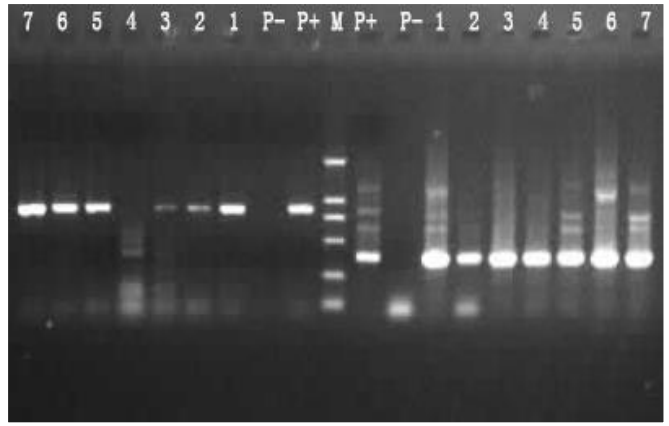
N gene targeted



i) OIE manual

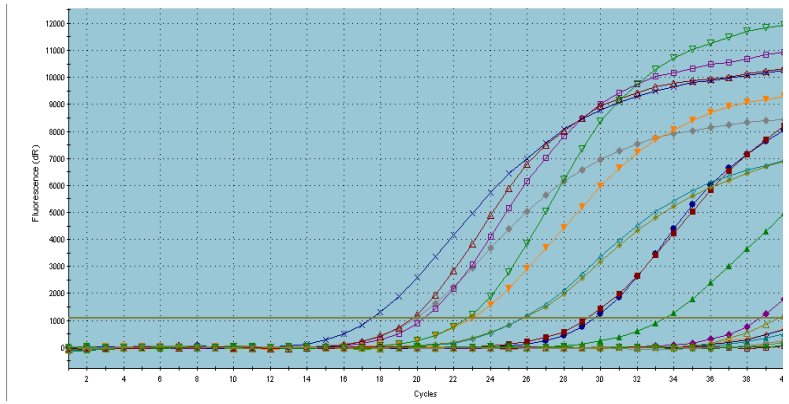


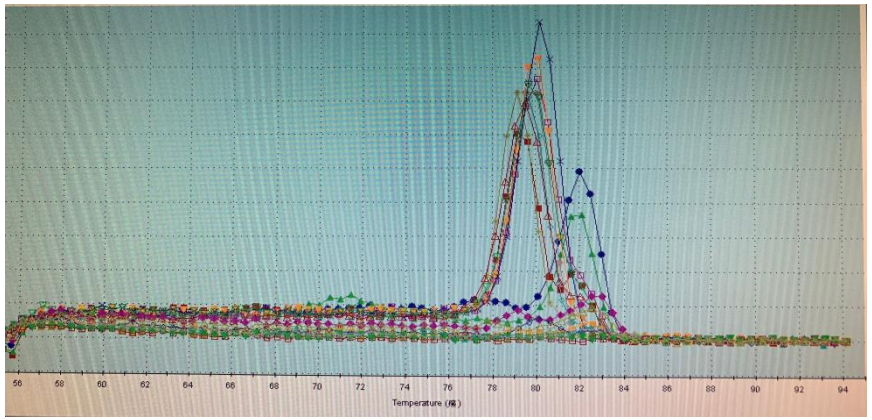
ii) Chinese RL method



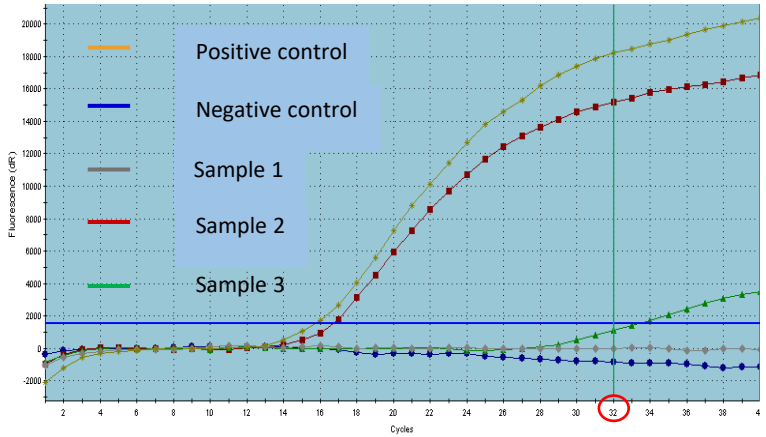
1.3.4 Real time RT-PCR

i) OIE manual： SYBR green method, Pan-lyssavirus





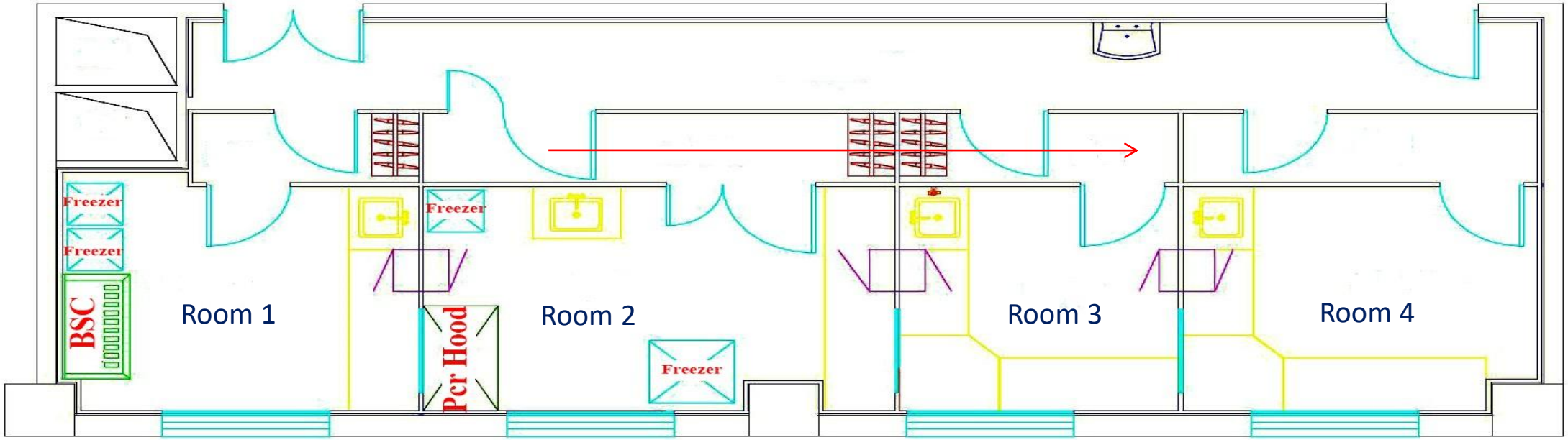
ii) Chinese RL method: Taq man method, RABV-specific



- Positive sample : 0<Ct<32 (sample 2)
- Negative sample: No Ct (sample 1)
- If Ct > 32 : Repeat assay for verifying (sample 3)

Room partition for PCR: Four-room principle

- Room 1: Reagent storage and pre-mixture prep
- Room 2: Prep of viral nucleic acid and PCR reaction
- Room 3: PCR
- Room 4:Gel running and documentation



Test of decomposed brain tissue

Comparison of 4 tests in detection of two decomposed samples

Day post decomposition	FAT*	RT-nPCR*	Tagman RT-PCR*	MIT*
1	+/+	+/+	+/+	-/-
2	+/+	+/+	+/+	-/-
3	+/+	+/+	+/+	-/-
4	+/+	+/+	+/+	-/-
5	+/+	+/+	+/+	-/-
6	+/+	+/+	+/+	-/-
7	+/-	+/+	+/+	-/-
8	-/-	+/+	+/+	-/-
9	-/-	+/+	+/+	-/-
10	-/-	+/+	+/+	-/-
11	-/-	+/+	+/+	-/-
12	-/-	+/+	+/+	-/-
13	-/-	+/+	+/+	-/-
14	-/-	+/+	+/+	-/-
15	-/-	+/+	+/+	-/-
16	-/-	+/+	+/+	-/-
17	-/-	+/+	+/+	-/-

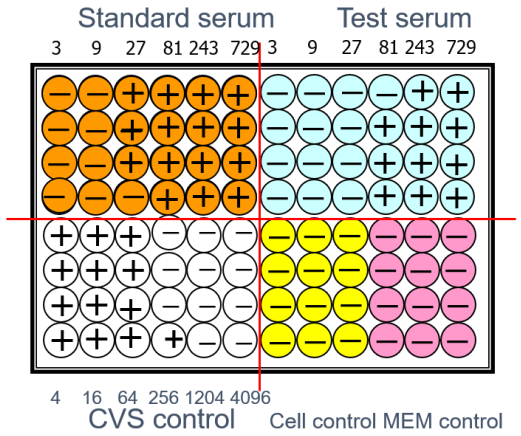
Note: “+”positive； “-”negative *TJ105 strain/CQQJ-09 strain

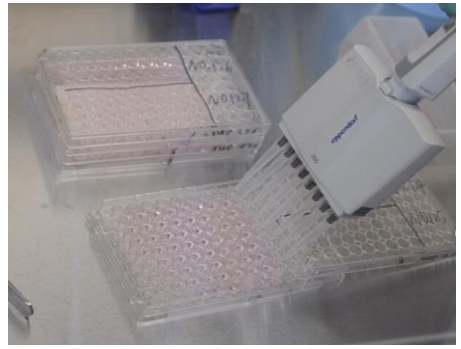
2. Serological Test

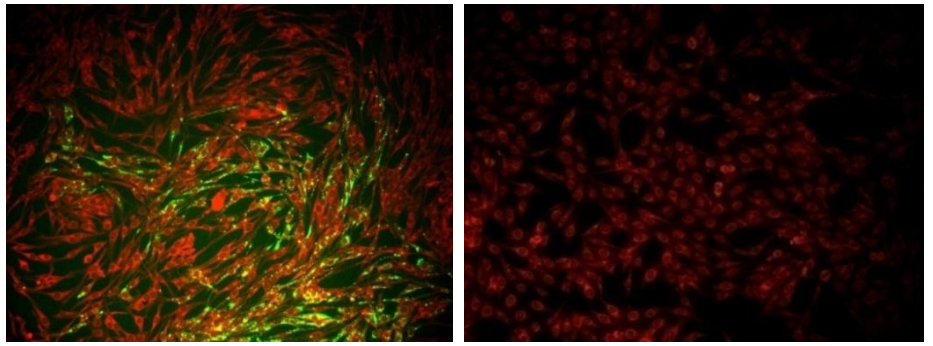
Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of immune response						
VN	n/a	+++	+++	n/a	n/a	+++
ELISA	n/a	n/a	+++	n/a	n/a	+++

2.1 Fluorescent Antibody Virus Neutralization (**FAVN**)

- OIE and WHO standard
- Prescribed test for international trade or travel of live animals
- Quantification of antibody level (0.5 IU/ml)
- Most widely used in the world
- Requisite for professional rabies laboratory
- Disadvantage： time-and-labor-consuming， BSL2, high cost.







- Formula to convert the log D_{50} value in IU/ml titre:

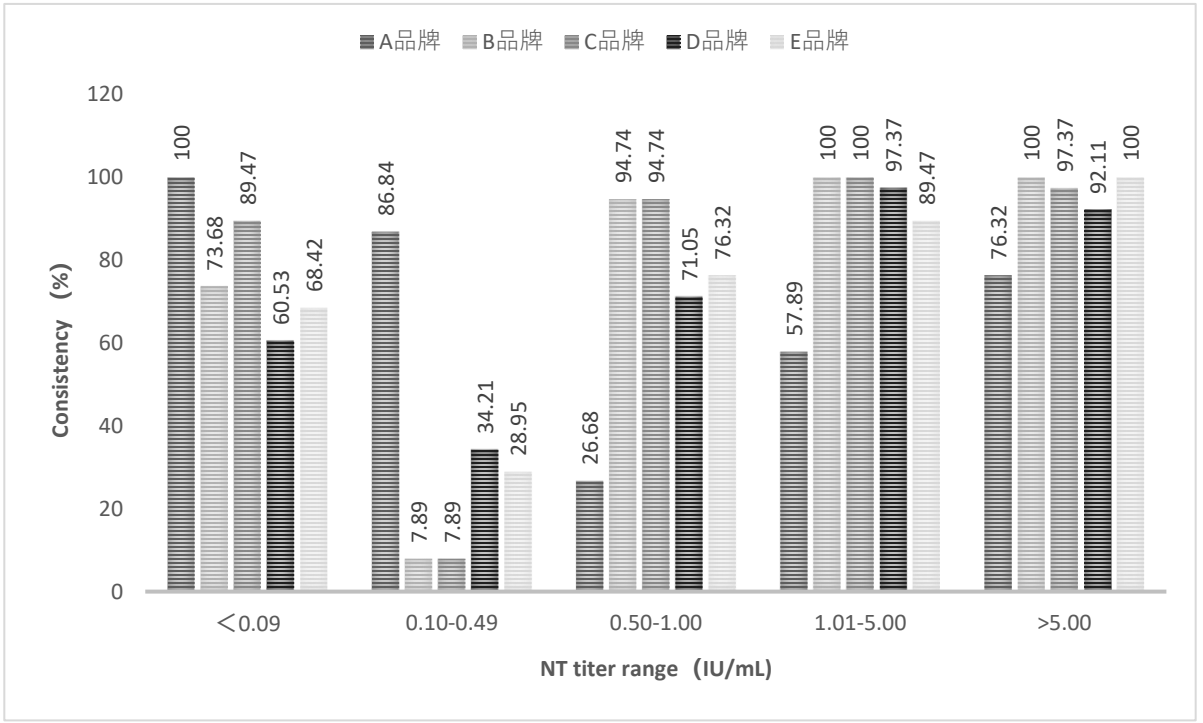
$$\text{Serum titre (IU/ml)} = \frac{[(10^{(\text{serum log } D_{50} \text{ value})}) \times \text{theoretical titre of OIE serum 0.5 IU/ml}]}{(10^{(\log D_{50} \text{ of OIE serum 0.5 IU/ml})})}$$

2.2 Ab ELISA

- OIE recommended
- Suitable to detect the herd immunity, not for titration of individual animals
- Commercialized
- Advantage: rapid, no live virus, large scale screening
- Disadvantage: not well correlates with VN, not for international trade or travel of live animals.

Brand	Sensitivity	Specificity
A	52.63%（60/114）	93.42%（71/76）
B	98.25%（112/114）	40.79%（31/76）
C	97.37%（111/114）	48.68%（37/76）
D	86.84%（99/114）	47.37%（36/76）
E	88.60%（101/114）	48.68%（37/76）

Comparison of 5 commercial kits with FAVN



Thank you for your attention!